

Evaluation of advanced stage maize hybrids for kernel zinc and kernel iron

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ABSTRACT

The present study evaluated 20 advanced stage maize hybrids to assess genetic variability, heritability and the association of kernel iron (Fe) and zinc (Zn) with grain yield. Significant variability was observed for agronomic traits, grain yield and kernel micronutrient concentrations. Cob length showed high heritability (0.84), while days to 50% anthesis, days to 50% silking, grain yield and kernel Fe exhibited moderate heritability, indicating scope for genetic improvement. Kernel Zn showed very low heritability (0.03), suggesting a strong environmental influence. Mean kernel Fe and Zn concentrations were 19.47 and 20.40 ppm, respectively. A significant positive correlation ($r = 0.68^{**}$) was observed between kernel Fe and Zn, indicating the feasibility of simultaneous improvement. Grain yield averaged 4.96 t/ha and showed a negative association with kernel Fe and Zn.

Keywords: *Advanced stage maize hybrids, Biofortification, grain yield, Heritability, kernel zinc and*

Micronutrient malnutrition, often referred to as “hidden hunger,” affects more than half of the global population, with women and children in developing countries being the most vulnerable. Diets deficient in bioavailable minerals and vitamins contribute to impaired cognitive development, reduced immunity in children and increased maternal mortality. Even mild deficiencies of essential micronutrients such as iron (Fe) and zinc (Zn) can have long-term adverse effects on human health, underscoring the urgent need for sustainable nutritional interventions. Biofortification, which involves enhancing the nutritional quality of staple food crops through plant breeding, has emerged as a cost-effective and sustainable strategy to combat micronutrient deficiencies. Unlike post-harvest fortification or dietary supplementation, biofortification targets the food system at its source by increasing the concentration and bioavailability of essential nutrients in the edible portions of crops. However, micronutrient concentrations are often inherently low in major cereal crops such as rice, wheat and maize, necessitating a thorough understanding of the genetic variability governing kernel micronutrient accumulation.

Maize (*Zea mays* L.), widely known as the “Queen of Cereals,” occupies a central role in global agriculture due to its high yield potential, adaptability,

and diverse end uses. In addition to being a staple food for millions of people, maize serves as a major component of livestock and poultry feed and as a raw material for starch, oil, biofuel, and processed food industries. In many developing regions, maize constitutes a significant proportion of daily caloric intake, making it an ideal target crop for biofortification efforts aimed at alleviating micronutrient deficiencies, particularly those related to Fe and Zn. Zinc and iron are essential micronutrients involved in numerous physiological and biochemical processes. Zinc functions as a structural or catalytic component of several hundred enzymes and plays a key role in metabolism, gene expression, immune function and cellular integrity, while iron is indispensable for oxygen transport, respiration and various enzymatic reactions. Despite their importance, deficiencies of both Fe and Zn are widespread in populations dependent on cereal-based diets with low micronutrient density and bioavailability.

In parallel with efforts to enhance grain yield, increasing kernel micronutrient concentration, especially Fe and Zn, has become a major breeding objective in maize. However, grain yield is a complex quantitative trait influenced by multiple agronomic components, and concerns remain regarding potential trade-offs between yield and micronutrient

concentration. Therefore, understanding the extent of genetic variability for kernel Fe and Zn, as well as their relationship with grain yield and other agronomic traits, is essential for developing nutritionally enhanced maize hybrids without compromising productivity. The first step in any crop improvement program is the assessment of genetic diversity in the available germplasm. Evaluating advanced stage maize hybrids for kernel Fe and Zn content provides valuable insights into the feasibility of simultaneous improvement of yield and nutritional quality. In this context, the present study aims to evaluate advanced stage maize hybrids for kernel zinc and kernel iron concentration and to examine their associations with grain yield, thereby contributing to biofortification-oriented maize breeding programs.

MATERIAL AND METHODS

During kharif 2024, a total of 20 maize hybrids along with two standard checks were evaluated at CIMMYT (Asia), ICRISAT Campus, Hyderabad, Telangana, located at 17°53'2" N latitude, 78°27'2" E longitude and 545 m above mean sea level. The experiment was conducted using an alpha-lattice design with two replications. All 20 hybrids, including the checks, were randomized into four blocks with a block size of ten. Each genotype was planted in a single 2-m row with a spacing of 60 cm × 20 cm. Recommended agronomic practices were followed to ensure uniform crop establishment and reliable phenotypic expression. Details of the different parameters studied were as follows. Data were recorded on days to 50% anthesis (DA), days to 50% silking (DS), plant height (PH), ear height (EH), cob length (CL), grain yield (GY), kernel iron (Fe) and kernel zinc (Zn) content. Observations on various pre and post-harvest parameters were recorded on five plants selected at random from each entry in each replication.

Determination of Kernel zinc and iron contents

Zinc and iron content were quantified using an Oxford Instruments X-Supreme 8000 spectrometer equipped with a Rhodium (Rh) X ray tube and a high resolution silicon drift detector. Measurements were conducted using sample cups prepared following the procedures described by Paltridge *et al.* (2012) and Guild and Stangoulis (2016), with a 4 µm Poly-4 XRF film sealing one end

of each cup. All cups were thoroughly cleaned and prepared prior to analysis to minimize cross-contamination. Approximately 20 g of sample was used for each measurement to ensure that the material met the infinite thickness requirement for EDXRF analysis (Paltridge *et al.*, 2012). Calibration was performed using the maize flour 2016 method, standardized for zinc and iron quantification in maize flour in HarvestPlus at ICRISAT. These calibration standards were obtained from CIMMYT–Mexico. Measurements were repeated in duplicates and mean Zn content were expressed in parts per million (ppm) on a dry weight basis.

Statistical Analysis

The linear mixed models were implemented using the lmer from package lme4 of R, employing the restricted maximum likelihood (REML) method to calculate BLUPs and estimate the variance components. For calculating the BLUPs and broad-sense heritability, all effects are considered random. A mixed linear model was fitted to partition the total phenotypic variance into its components due to genotype, genotype × environment interaction and residual effects using REML algorithm in META-R v6.0. The significance of the genotype variance (σ_G^2) was determined through a likelihood ratio test (LRT) comparing the restricted log-likelihoods of a full model (with genotype effect included) and a reduced model (excluding the genotype term). The resulting p-values indicated whether the observed genetic variance differed significantly from zero. Significant p-values ($P < 0.05$) implied the presence of substantial genetic variability among genotypes across environments.

RESULTS AND DISCUSSION

In the present study, 20 maize hybrids along with two checks (NK30 and VH18221) were evaluated. The analysis was carried out using Meta-R to estimate BLUP values and variance components, enabling the assessment of genetic performance and variability among the hybrids (Table 1). The BLUP estimates for grain yield, yield-related traits and kernel zinc and iron content of the 20 advanced-stage maize hybrids are presented in Table 2. The significance of genotypic variance indicates whether the hybrids showed meaningful genetic differences for each trait. In this study, genotypic variance was significant for days to 50% anthesis (DA) and cob length (CL). For

Table 1: Variance components and descriptive statistics for 20 advanced stage maize hybrids

	DA	DS	PH	EH	GY	CL	Fe	Zn
Heritability	0.66	0.63	0.00	0.42	0.63	0.84	0.64	0.03
Genotype Variance	0.57*	0.55	0.00	35.80	1.30	3.06**	2.25	0.15
Residual Variance	0.60	0.65	122.50	97.33	1.54	1.16	2.52	11.41
Grand Mean	56.63	58.15	216.71	106.35	4.96	16.52	19.47	20.40
CV	1.36	1.38	5.11	9.28	25.02	6.53	8.15	16.56
Genotype significance	0.04	0.09	1.00	0.30	0.09	0.0003	0.07	0.96
Minimum	56.17	57.63	216.71	96.84	3.22	14.16	17.59	20.28
Maximum	58.20	59.34	216.71	112.72	6.64	18.50	21.28	20.48

days to 50% silking (DS), grain yield (GY) and kernel iron (Fe) content showed marginal significance, reflecting moderate genetic variation. In contrast, plant height (PH), ear height (EH) and kernel zinc (Zn) content did not show significant genotypic variance, indicating limited detectable genetic differences among the hybrids for these traits.

Cob length showed high heritability of 84%, demonstrating strong genetic control and suggesting that this trait can be effectively improved through breeding. Similar results were reported by Sumalini *et al.* (2012) and Tengan *et al.* (2012). Days to 50% anthesis (0.66) and days to 50% silking (0.63) showed moderately high heritability, suggesting that these traits can be improved effectively through selection. Similar results were reported by Reddy *et al.* (2022). Grain Yield (0.63) also exhibited moderate heritability, indicating that although environmental effects are present, genetic variation contributes substantially to yield differences among hybrids. Similar studies were reported by Sesa *et al.* (2016) and Jilo *et al.* (2018). The analyzed traits exhibited varying ranges across the evaluated hybrids. Days to 50% anthesis and days to 50% silking had grand means of 56.63 and 58.15 days, with range of 56.17-58.20 and 57.63-59.34 days, respectively. Similar studies were reported by Patil *et al.* (2016). Plant height and ear height averaged 216.71 cm and 106.35 cm respectively, while cob length (CL) averaged 16.52 cm (14.16-18.50 cm). Similar studies were reported by Bartaula *et al.* (2019).

Kernel iron (Fe) and zinc (Zn) contents had means of 19.47 and 20.40 ppm. Kernel iron content showed moderate heritability (0.64), indicating that selection for higher Fe levels can be effective. In contrast, kernel zinc content had extremely low heritability (0.03), indicating that environmental variation predominates and that genetic differences

for Zn are minimal or largely undetectable. Comparable results were reported by Pixley *et al.* (2011), who observed Fe content ranging from 12.9 to 26.5 ppm and Zn content from 19.3 to 30.9 ppm in maize hybrids. The relatively narrow range observed for Zn in the present study suggests that the evaluated lines exhibited broadly similar Zn levels, with an average of approximately 20.4 ppm. Qin *et al.* (2012) reported moderate heritability estimates (<0.60) for kernel Fe content.

Kernel iron and zinc content showed significantly high positive correlation of 0.68** indicating both can be improved together in the breeding programme. Several studies have consistently documented a significant positive association between kernel Fe and Zn concentrations (Lungaho *et al.*, 2011). This relationship may be attributed to genetic linkage between loci controlling micronutrient accumulation or to pleiotropic effects of genes involved in metal uptake and transport. Notably, a large number of genes encode metal transporter proteins, some of which are capable of transporting multiple metal ions, thereby influencing the concurrent accumulation of Fe and Zn in kernels (Qin *et al.*, 2012).

Grain yield (GY) showed a mean of 4.96 t/ha, with a range of 3.22-6.64 t/ha. Similar studies were reported by Magar *et al.* (2021). A total of 10 genotypes (50%) recorded values equal to or above the overall mean, indicating the presence of considerable variability for grain yield within the evaluated set. CLTHWZN21544 (6.64 t/ha) showed superior yield when compared with the check NK30 (6.47 t/ha). Grain yield is a key trait determining the acceptance of a cultivar by the farming community. Therefore, understanding its genetic relationship with kernel micronutrient concentration is essential for biofortification breeding. In the present study, grain

Table 2. BLUP-based estimates for grain yield and its component traits along with kernel zinc and iron content in 20 advanced-stage maize hybrids

Pedigree	BLUP Values							
	DA	DS	PH	EH	GY	CL	Fe	Zn
CLTHWZN19514	56.88	58.35	216.71	101.87	4.94	15.67	19.42	20.46
CLTHWZN19518	56.88	58.35	216.71	108.87	4.74	17.35	21.28	20.45
CLTHWZN19520	56.81	58.38	216.71	112.6	3.7	16.07	20.64	20.44
CLTHWZN19523	56.24	57.63	216.71	107.77	3.22	16.18	NA	NA
CLTHWZN20525	56.61	58.68	216.71	104.38	5.59	16.47	20.78	20.38
CLTHWZN19502	58.2	59.34	216.71	110.57	3.71	15.71	20.12	20.48
CLTHWZN21526	56.56	57.65	216.71	107.01	5.07	17.28	17.82	20.34
CLTHWZN21529	57.3	59.12	216.71	104.88	4.3	14.27	19.79	20.39
CLTHWZN21531	56.17	57.95	216.71	107.47	5.41	14.37	17.59	20.28
CLTHWZN21537	56.17	57.66	216.71	109.7	5.58	14.16	20.06	20.46
CLTHWZN21538	56.26	57.84	216.71	106.4	4.64	18.5	18.56	20.44
CLTHWZN21544	56.65	57.86	216.71	112.72	6.64	15.53	18.27	20.33
CLTHWZN21548	57.11	58.28	216.71	105.64	5.17	17.69	19.42	20.38
CLTHWZN21549	56.23	57.77	216.71	104.21	4.99	16.13	20.57	20.38
CLTHWZN17103	56.5	58.29	216.71	107.58	4.28	15.42	18.1	20.37
CLTHWZN17113	56.44	57.95	216.71	96.84	4.85	16.17	19.7	20.38
CLTHWZN18209	57.44	58.91	216.71	106.28	4.48	16	20.83	20.47
CLTHW16138	56.72	58.49	216.71	104.99	5.21	18.24	19.23	20.35
Internal Check-1 VH18221	55.3	57.13	216.71	102.16	6.14	19.25	NA	NA
check 2-NK30	56.23	57.4	216.71	105.05	6.47	19.87	18.3	20.36

yield exhibited a negative association with both kernel zinc and kernel iron contents. Increased carbohydrate content in the grain of high yielding genotypes possibly dilutes the content of the micronutrients (Banziger and Long 2000). The magnitude and direction of this association are known to be influenced by environmental conditions (Šimić *et al.*, 2009), as well as by the nature and genetic background of the germplasm evaluated (Pixley *et al.*, 2011). Similarly, Long *et al.* (2004) observed a negative correlation between grain yield and kernel Fe. Baxter *et al.* (2013) reported a weak negative association between kernel weight and kernel Fe and Zn concentrations. Despite the generally negative relationships reported between grain yield and kernel micronutrients, several studies suggest that it is feasible to identify and select maize germplasm combining high grain yield with elevated kernel Fe and Zn concentrations (Banziger and Long, 2000).

CONCLUSION

The present study revealed substantial variability among advanced stage maize hybrids for

agronomic traits, grain yield and kernel micronutrient concentrations. Moderate to high heritability estimates for cob length, flowering traits, grain yield and kernel iron content indicate effective scope for genetic improvement through selection. In contrast, the very low heritability observed for kernel zinc suggests a strong environmental influence and highlights the need for multi-environment evaluation. The significant positive correlation between kernel iron and zinc concentrations indicates the possibility of simultaneous improvement of both micronutrients. Although grain yield showed a negative association with kernel iron and zinc, demonstrates that yield improvement and nutritional enhancement are not mutually exclusive. Overall, the findings support the potential of advanced stage maize hybrids as promising genetic resources for iron biofortification and emphasize the importance of integrated breeding approaches to develop nutritionally enriched, high-yielding maize cultivars.

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